Towards a greater understanding of the presence, fate and ecological effects of microplastics in the freshwater environment

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CHAPTER 7

Discussion

1. Microplastics: a rapidly expanding field of research

This PhD thesis has been written during a time when the state of the knowledge of microplastics has been rapidly expanding, with multiple articles now published on the topic every week. This research effort has corresponded with an increased public awareness, often under the impression that microplastics are inherently toxic and harmful within the environment, despite the lack of clear evidence to support this view. It is unusual for such keen interest in a topic to precede scientific evidence, and as such, research has had to work fast to provide answers to the basic overarching questions posed by the public such as 'how much plastic is in the environment?' and 'is this a problem for ecosystem and human health?'. Despite this awareness of the potential problems associated with plastic pollution, the manufacture and usage of plastics continues to grow and the amount of plastic waste within the environment is predicted to increase (Geyer et al., 2017; Jambeck et al., 2015).

Chapter 2 was published at the time when this proliferation in research and understanding, especially of microplastics in freshwaters, was just beginning. This review comprehensively examined the current state of the knowledge of microplastics in freshwater and terrestrial environments, identifying the major knowledge gaps and the significant questions that needed to be addressed to develop our understanding in this area. This review highlighted the limited knowledge of microplastics in freshwater systems compared to the marine environment, and demonstrated where comparisons can be made between the two systems, to inform our understanding and help develop forward-thinking research questions. It also emphasised the lack of knowledge of microplastics in terrestrial environments, despite the widespread manufacture and use of plastics on land, including agricultural practises, in addition to industrial and domestic use. This made particularly clear the challenges involved in microplastics research, including the lack of method standardisation for environmental surveys, which hinders comparability between data and will be essential to address in the research field going forward.

There are contradictory results across studies on both the ecotoxicological effects of microplastics, and on the effects of associated chemicals (Beckingham and Ghosh, 2016;

Koelmans et al., 2016; Rochman et al., 2013c). This is partly due to the heterogeneous nature of the particles covered by the term 'microplastics' which can refer to particles of any polymer type, shape and size (below 5 mm) (Rochman et al., 2019), in addition to the variation in species' sensitivity to physical and chemical stressors (Adam et al., 2019). This makes it extremely difficult to predict the effects of microplastics on species and ecosystems. Such heterogeneity must, therefore, be taken into account when designing toxicity studies, in order to ensure that future studies will enhance our understanding of how these varied particle characteristics (e.g. size, shape, polymer) will influence their hazard. This must also be considered across a range of ecologically important species in order to determine which species and ecosystems may be most at risk from microplastic exposure.

Writing the review helped to clarify a number key questions considered in this thesis and my future research including: 1. What are the sources of microplastics to freshwater environments, how are microplastics transported and do they accumulate within freshwater sediments? 2. Do freshwater organisms interact with/ingest microplastics and can this be linked to environmental or physiological factors? 3. Does acute exposure to microplastics lead to eco(toxico)logical effects? 4. Do microplastics mediate the effects of different hydrophobic organic chemicals on toxicity, bioaccumulation and the microbiome? These questions have been addressed to an extent in the following thesis chapters, advancing our understanding of these key research challenges.

2. Microplastics in UK rivers – sources and ecological interactions

Before the publication of **Chapters 3 and 4**, no studies had been carried out to investigate the presence of microplastics within UK freshwater systems, and there was no knowledge of ingestion by UK freshwater organisms. This was a critical gap in our understanding of microplastic presence, distribution and ecological interactions, and therefore the first two studies carried out as part of this PhD thesis intended to address these two questions. As one of the most economically and commercially significant rivers in the UK, the River Thames catchment was chosen as our study system for both the environmental and the ecological studies.

It is understood that microplastics will derive from a wide range of sources and will be transported to the freshwater environment in different ways including via land run-off, drainage systems or sewage effluent input. The latter represents a variety of sources that will be

commonly released to wastewaters including microfibres released from synthetic fabrics when laundered and microbeads from cosmetic products (Ziajahromi et al., 2016). This is the easiest route of input to quantify and characterise based on known volumes of sewage treated, known populations contributing to sewage treatment systems within specific areas, and existing models to correlate these data with river flow data to estimate riverine effluent concentrations (Williams et al., 2009). For **Chapter 3**, sewage effluent input and population density were therefore chosen as predictors of microplastic presence in sediments at four sites in the River Thames basin (UK) Microplastics were found at all four sites. One site had significantly higher numbers of microplastics than other sites, average 66 particles 100 g^{-1}, 91% of which were fragments. Contrary to our hypothesis, this was not the site receiving the highest concentrations of effluent, but it was a site downstream of a storm drain outfall receiving urban runoff. Many of the fragments at this site were determined to be derived from thermoplastic road-surface marking paints, showing a clear and unimpeded pathway directly from the road surface to the river sediment. Road marking paints as a source of microplastics to the freshwater environment had not previously been described, and therefore these data were a significant contribution to the field of microplastics research. At the remaining three sites, fibres were the dominant particle type, as is the case in the majority of environmental microplastic studies (Barrows et al., 2018; Carr, 2017). These were present even at the sites with little sewage influence, further suggesting that effluent may not always be the dominant route of entry for microplastics to the riverine environment. For example, recent research has highlighted the likely high contribution of atmospheric transport and deposition of particles to regions where inputs may not otherwise have been expected to be significant (Allen et al., 2019).

A study has since been carried out which found that microbeads dominated the microplastics found within sediments at multiple rivers around Manchester, UK (Hurley et al., 2018a). This is likely due to the size range analysed, as the majority of particles observed by Hurley et al. (2018a) were < 1 mm, smaller than those analysed in our study. These results also show the extent to which the methods used for sampling and analysis may influence the result seen. With the results presented in Chapter 3, it is not possible to determine whether microbeads were present, given that the majority of these are likely to be < 1 mm, and therefore they would not been observed using our techniques. Additionally, their study showed the influence of intermittent weather events on particle concentrations, with concentrations in sediments significantly reduced following flooding (Hurley et al., 2018a). This highlights the importance of reporting the environmental conditions at the time of sampling, and undertaking time-series

sampling where possible. All rivers will experience periods of high and low flows, and therefore this is likely to be a significant factor influencing microplastic concentrations within riverine environments. Given that our study was a 'snapshot' in time, having been carried out on a single sampling occasion, it would be recommended for future studies to include temporal variation to better understand the dynamics of microplastic presence in sediments and surface waters.

While it has since been further acknowledged that road paints will be a source of microplastics to freshwater systems, few subsequent studies have shown evidence for road paints within environmental samples. Instead interest has shifted to the likely high contribution of tyre-wear particles to the number of microplastic particles in freshwaters (Abbasi et al., 2017; Boucher and Friot, 2017; Kole et al., 2017; Verschoor et al., 2016). However, paints as plastic composites remain of interest, especially within the marine environment, given their widespread use on boats and ships. Indeed paint particles have been found in marine sediments and surface waters (Chae et al., 2015; Reddy et al., 2006). It is important to note that such particles derived from maritime activities may pose the additional hazard of being derived from antifouling paints with biocidal properties, containing high levels of metals such as copper and zinc (Brennecke et al., 2016).

Following the identification of microplastics with the River Thames basin (UK), it was decided to investigate ingestion of microplastics by fish within this habitat. **Chapter 4** provided the first study of ingestion of microplastics by the freshwater fish (the common roach, *Rutilus rutilus*) within the River Thames. In combination with existing relevant literature (Andrade et al., 2019; McGoran et al., 2018; Sanchez et al., 2014), this study provided an insight into the factors influencing fish exposure and ingestion within riverine environments. This study aimed to link fish ingestion to environmental exposure (i.e. increased distance downstream from the source of the river was expected to increase exposure) and physiological factors (fish length and gender). Microplastics were found within the gut contents of roach from six out of seven sampling sites. Of the fish sampled, 33% contained at least one microplastic particle. This corresponds with a number of other studies which have found similar proportional contamination of fish with microplastics: 28% of fish sampled within the Adriatic Sea (Avio et al., 2015b), 32% within the Thames Estuary and the Clyde (McGoran et al., 2018), 36.5% in the English channel (Lusher et al., 2013). The majority of particles were fibres (75%), with fragments and films also seen (22.7% and 2.3%, respectively). This also corresponds to other

studies which found fibres to be the dominant particle type: 68% (Lusher et al., 2013), 83% (Steer et al., 2017) and 88% (McGoran et al., 2018).

Despite some similarities of our data to other studies, it must be noted that results across studies are also extremely variable in many respects. For example, while the particle types found by Steer et al. (2017) were dominated by fibres and were, therefore, proportionally related to what we found, contrary to our results they found only 2.9% of fish (larvae) had ingested microplastics. In our study, larger fish were found to be more likely to ingest a predicted maximum number of particles at a given location (based on quantile regression) than smaller fish. Female fish were more likely to ingest the predicted maximum number of particles than male fish. To our knowledge, gender-specific differences have not been highlighted in other microplastic ingestion studies. This suggests that intra- or interspecific factors such as size, gender or life stage may have a significant influence on feeding habits and thus ingestion of microplastics. Such factors are not currently well understood or described and this may explain many of the differences seen between studies. Further research on how organism physiology will influence ingestion is therefore recommended.

Where possible, ingestion studies should also seek to quantify surrounding environmental microplastic concentrations, for example within the water column, as this will enable an understanding of the link between exposure and ingestion. Such data providing evidence of direct links between exposure and ingestion are surprisingly scarce. Understanding of fish exposure to microplastics could be furthered by investigating trophic interactions to determine the likely dominant routes of microplastic uptake across different species, relationships which, to date, have been little studied within freshwater systems (Chae et al., 2018; Windsor et al., 2019b). Such knowledge will be valuable in informing future studies of microplastic impacts on fish health, and the wider ecological and economic implications of this (Lusher et al., 2017).

3. Challenges and recommendations for method development

Methods for microplastic sampling, sample processing and analysis have developed considerably in the last few years, even since the inception of this PhD. At the time of carrying out the analytical work for **Chapters 3 and 4** it was commonplace to visually identify and quantify microplastics by eye using a binocular microscope, and then verify this identification using spectroscopy, methods focussed on and optimised within these chapters. Similarly, the minimum size of particle commonly identified was in the hundreds of microns scale. More

recently, studies are increasingly moving towards more automated and technologically advanced methods, for example using fluorescence staining and image analysis, FTIR mapping, or mass quantification using thermo-analytical methods. Such methods eliminate bias and allow for the identification of much smaller particles down to tens or even 1 µm (Cabernard et al., 2018; Erni-Cassola et al., 2017; Simon et al., 2018). However, the more manual methods are still widely utilised by researchers who are using relatively recently-published literature for guidance.

With respect to the reporting of microplastics data, these may be reported in different units *i.e.* either by mass or number of particles, leading to difficulties when attempting to compare results between studies. To some extent the units reported are reliant on the methods used – manual and spectroscopic methods rely on counting and characterising individual particles, whereas chemical analysis techniques produce results by mass of polymer. This has implications for interpretation, comparison and utilisation of data across different studies. Where extrapolation is possible, for example using quantitative data on number, size and polymer type of particles to also estimate a mass, this is advisable to enable greater comparability between studies. Due to the fast rate of knowledge expansion and method development in this field, it must be acknowledged that some lag is inevitable if allowing all researchers to 'catch up'. In fact, while methods have significantly improved in recent years, it is recognised that due to the continually improving analytical capabilities for microplastic detection, quantification and polymer analysis, method development is likely to continue at pace for some time.

The utilisation of different techniques, methods and units for collection and reporting of microplastic data is a widely-recognised hindrance to comparability of data between studies. While there is a call for development of protocols (including by the British Standardisation Committee, BSI, and the International Standardisation Organisation, ISO), based on equipment accessibility and also sample heterogeneity, it may not be reasonable to suggest that all studies adhere to a strict standard protocol. Further, the technical capabilities will likely continue to progress with the development of analytical equipment that is fit for purpose, and therefore standard methods determined now could be outdated within a few years. As such, care must be taken to regularly evaluate and update any written protocols and recommendations that may be put into place. Despite this, a few key recommendations can be made now, and should be agreed across the research community. As a minimum, all methods should be reported step-by-step, including all specifications of sampling or analytical equipment used, the time/depth/volume/weight of sample taken, particle size analysed (minimum and maximum),

how subsamples were selected, any software parameters for undertaking data analysis and any calculations used to extrapolate to concentrations. It is essential to detail any uncertainty or ambiguity in microplastic analysis, for example, where reporting false positives or negatives may significantly influence results.

A critical consideration for high-quality analysis of environmental samples is that of contamination controls and blanks. Since the early microplastic studies it has been recognised that samples may become contaminated in the lab by airborne particles, although often little in the way of contamination control is implemented (Hidalgo-Ruz et al., 2012; Koelmans et al., 2019). In the past, at most this may have consisted of a petri dish left open to the air and later examined for contamination. However, recently it has been noted that more rigorous controls are necessary, including blank process controls to account for contamination throughout all sample collection and processing steps. Recent analyses have suggested that the majority of studies to date have not carried out sufficient controls to ensure the reliability of the data collected (Hermsen et al., 2018; Koelmans et al., 2019). It has also been recognised that plastic laboratory equipment in itself may shed particles and contaminate samples, therefore glassware is recommended over plastic wherever possible. It is also suggested to wear natural fabrics during sample collection and processing, although this is not always practical (especially in the case of fieldwork). Within the lab, a cotton lab coat should be worn to cover clothing (Woodall et al., 2015).

It is also highly recommended to carry out positive controls to account for any particle loss during processing, as is the case with chemical analyses, to determine what proportion of particles are actually being recovered from the matrix (Koelmans et al., 2019). It is possible that many studies provide an underestimation of particles as a result of ineffective recoveries based on the extraction procedures used. This is especially the case for very small particles which may have been missed due to filtering or sieving carried out as part of the sample collection or processing methods (Hurley et al., 2018b).

A collaborative effort across the research community to adhere to such recommendations will allow for the utilisation or comparison of these methods in future studies as appropriate for the question being asked, and thus allow for the harmonisation, rather than the standardisation of methods (Rochman et al., 2017). Therefore, while not always directly comparable to other studies, resulting data can be compared with known limitations. If repeating the studies published within this thesis, contamination controls would undoubtedly be more rigorous and

recovery standards would have been implemented. However, these studies were carried out in line with recommendations at the time.

It is especially important to bear in mind that, when considering recommendations for future microplastic research, analytical techniques may not be of interest simply to academics, but ultimately to governments who may wish in the future to regulate microplastics across a range of systems. Furthermore, the industries that will be required to monitor their contributions in line with these regulations will need to keep up to date with methodological recommendations, in anticipation of needing to take action. Without comparable and repeatable methods, reliable scientific evidence to inform regulations will be difficult. Given this, and for the inclusion of developing countries in progressing the research, it is important that simple, time-efficient and cost-effective methods remain valid for the analysis of microplastics, providing sufficient information on these methods and the limitations of the study are provided in any published reports. For non-academics, or those with limited resources, it may not always be necessary to identify every particle by polymer type, shape and size if simply quantifying microplastics is the desired outcome. However, contamination controls must be in place, quality assurance must be met (e.g. accounting for particles found in blank samples), polymer confirmation is needed to eliminate false positives, even if only on a subsample, and reporting must be clear. Based on recent methodological developments, going forward it would always be recommended to use methods which eliminate bias (rather than identification by eye), the most simple of which is fluorescent staining combined with image analysis (Erni-Cassola et al., 2017; Maes et al., 2017).

4. Microplastic toxicity and effects on chemical bioavailability

There is still a degree of uncertainty as to whether or not microplastics influence the bioavailability and toxicity of hydrophobic organic chemicals. The variability in results between studies is largely likely to be due to the complexities of comparing different polymers, chemicals, organisms and environmental matrices, all of which will influence chemical associations and dynamics. It is too crude to consider simply ‘microplastics’ and ‘hydrophobic chemicals’ as single contaminants due to the great diversity among these materials (Rochman et al., 2019).

Given that microplastics and chemicals within the environment will rarely, if ever, occur independently, it is essential that we understand how these mixtures may differently influence

species and ecosystems compared to individual pollutant exposures, as are usually carried out within the lab. For this reason, within the studies detailed in **Chapters 5 and 6**, two different polymer types were selected, with differing densities (low = polystyrene and high = nylon) alongside two particular types of chemicals with uses that could lead to their release to the environment (1. pesticides: dimethoate and deltamethrin, and 2. flame-retardants: PBDEs). As test organisms two different species were chosen, *Daphnia magna*, a keystone species within pelagic food webs and *Lymnaea stagnalis,* a benthic feeder. Despite the intentional variability of chemicals, polymers and organisms within and between these studies, the overall outcome was the same: microplastics had no influence on chemical toxicity or bioavailability. Microplastics did not influence the toxicity of chemicals (*Daphnia magna* exposed to polystyrene and pesticides, **Chapter 5**) or the accumulation of chemicals (*Lymnaea stagnalis* exposed to nylon and PBDEs, **Chapter 6**). This result is in line with a number of other recent studies which found that microplastics did not influence bioavailability or toxicity of hydrophobic organic chemicals (Ašmonaitė et al., 2018; Devriese et al., 2017). Due to the relatively low mass of microplastics within the environment, a greater proportion of hydrophobic chemicals will associate with natural organic and inorganic particulate matter. Under natural environmental conditions, microplastics are therefore likely to have a negligible effect on the bioavailability of associated chemicals (Bakir et al., 2016; Koelmans et al., 2016).

Comparing the results in **Chapters 5 and 6** to previous studies on microplastics and associated chemical toxicity in this field highlights the variability between the results of different studies, which are highly dependent on the organisms exposed, the particles used and the exposure conditions. For example, the PBDE exposure conditions in **Chapter 6** were very different in many ways to a study carried out by Rochman et al. (2013c), who found that the presence of microplastics significantly influenced the bioaccumulation of PBDEs in fish. When considering chemical accumulation, a different organism would likely have accumulated PBDEs (and different congeners) differently. For example, some organisms are more likely to bioaccumulate hydrophobic chemicals than others, based on lipid content (Gobas, 1993; Mackay and Fraser, 2000). It could be valuable to evaluate such responses to microplastics in association with other indicators of organism sensitivity where available. For example, existing toxicity data of common chemical pollutants may help to understand the underlying mechanisms and specific traits which can influence sensitivity, including inter- and intraspecific differences such as metabolism or life stage (Baas and Kooijman, 2015; Mohammed, 2013). Such understanding of differential organism responses may help in

predicting susceptibility to harm by microplastics. However, given that microplastics pose a combined chemical and particulate threat (Rochman, 2013), it should also be acknowledged that responses to microplastics may differ greatly to other stressors, and thus continued research into these effects under varying conditions is essential. In addition to the organism itself, the particle properties, including size, shape and polymer type will significantly influence the likelihood of ingestion and possible hazard. Finally, the route and duration of exposure can also significantly influence the effects seen, for example, PBDEs spiked directly into the water may have behaved differently and been differently bioavailable compared to the sediment-based exposure we carried out. It is therefore critical that we consider these factors when interpreting data, as they have implications for our understanding of the effects of microplastics and hydrophobic chemicals, both individually and in combination.

It is not feasible to experimentally test all the possible permutations of species, particles and environmental conditions, therefore it would be valuable to learn from other areas of ecotoxicology which have considered this challenge. For example, a traits-based approach to understanding sensitivity has been recommended for nanotoxicology research, based primarily on understanding how organism morphology and physiology will determine sensitivity. This involves extrapolating known interactions between organisms, particles and the environment to enable prediction of how these factors will interact across a wider range of conditions, and the subsequent likelihood of harm (Song et al., 2011). Alternatively, read-across models may take a different approach by combining knowledge of the physico-chemical properties of particles and their interactions with the environmental matrix to predict exposure (Gajewicz, 2017; Quik et al., 2018). While potentially valuable for making predictions in future microplastic research, both of these approaches rely on sufficient availability of data, which for microplastics is still lacking.

5. Microbiome response to microplastics and flame-retardants

One aspect of the potential biological effects of microplastics that has been little studied is that of the microbiome. It is known that the gut microbiome is susceptible to perturbation as a result of chemical or physical stress (Moya and Ferrer, 2016). The limited response of the microbiome to microplastics and PBDEs observed in **Chapter 6** was an unexpected finding, as most studies investigating the effects of PBDEs and microplastics (independently) have found significant effects relating to an increased availability of hydrophobic organic chemicals

(Chen et al., 2018; Zhu et al., 2018a). However, these previous studies ran for a minimum of seven days whereas ours was a 96 hour exposure, implying that exposure duration is likely to be highly significant. It is understood that acute and chronic microbiome responses will be different (Shade et al., 2012), with some communities resilient to short-term or 'pulse' perturbations (Sommer et al., 2017). Therefore, it is likely to have been the case that in this study, the pulse exposure was insufficient in duration to induce a change in the microbial diversity or community composition. Pulse exposures are highly relevant in an environmental context, as inputs and chemical concentrations within the environment will fluctuate enormously, especially within highly dynamic environments such as rivers. As such, organisms are rarely likely to be exposed to consistent concentrations of chemicals (Handy, 1994; Reinert et al., 2002). However, it must be considered that microplastics will not readily degrade and therefore can accumulate within environmental sinks (Browne et al., 2011; Corcoran et al., 2015; Turner et al., 2019). In a relatively enclosed and undisturbed environment where microplastics can accumulate, there is therefore the potential for organisms to be chronically exposed. This highlights the need to consider how chronic low-level exposures may differ from acute exposures when trying to interpret organism and ecosystem responses to microplastic pollution. Further, differences between organism microbiome responses will be species-specific, for example relating to feeding habits and gut retention time. This therefore requires linking our understanding of environmental concentrations and conditions (exposure) to the effects seen in laboratory exposures (hazard).

6. Implications and impact of this research

It is clear that microplastics are everywhere within the environment. While there is a lot of available information on microplastics in the oceans, there is still a lesser understanding of microplastics in freshwater and terrestrial systems, despite the recent increase in research. Many of the knowledge gaps and research questions outlined in **Chapter 2** still remain. Rather than focussing on simple presence and abundance studies, in future studies it will be important to investigate the factors influencing microplastic behaviour and fate, for example, biofouling, weathering and degradation, to better understand where and why microplastics accumulate. While flux and transport models are becoming increasingly more commonplace to assess the volumes and transport of microplastics within the environment, it is essential that data are available with which to parameterise and validate these models, across a variety of different

environmental scenarios. For example, it is recognised that lakes can act as sinks for microplastics. Using sediment cores, this can provide stratigraphic evidence of microplastic accumulation that can be directly related to temporal trends in microplastic deposition (Turner et al., 2019). This is in contrast to riverine environments where flow conditions can lead to the rapid mobilisation of particles (Horton and Dixon, 2018). Identifying local sources, inputs and hotspots at specific sites (for example road marking paints as identified in **Chapter 3**), in addition to the types and characteristic of particles found, will provide new information to inform models. This is especially important given that some models may have been originally developed to predict chemical concentrations and transport, while microplastics by their nature will behave very differently to the majority of chemical pollutants. Such field data will, therefore, enable an improved understanding of the importance of these localised inputs to the more widespread movement of microplastics within river systems. If inputs or accumulations are found to be significant, for example from urban drainage, this will have implications for civil engineering and town planning decisions, helping to inform the cost-benefit assessment of materials used within urban settings, in addition to the design and regulation of such systems. This evidence will therefore contribute to better-informed decision-making for policy and industrial practices based on a greater knowledge of microplastic sources, environmental inputs, and mechanisms of transport, allowing for targeted industry and location-specific mitigation measures to be implemented.

Knowing that microplastics are widespread throughout the freshwater (and wider) environment and understanding hotspots of contamination, then developing a greater knowledge of organism interactions with microplastics, and the associated hazard, is critical for determining potential ecological effects. It is clear from the findings in **Chapter 4** that variations in ingestion between individuals can be highly intra-specific. A greater understanding of the physiological factors influencing ingestion within and between species is therefore essential, alongside a sound understanding of the environmental factors influencing exposure.

The studies detailed within **Chapters 5 and 6** aimed specifically to investigate the influence of microplastic presence on chemical toxicity and accumulation, therefore we used very high concentrations of microplastics in order to maximise the likelihood of interactions between the organisms, microplastics and chemicals. Concurrently it was also possible to observe the response of organisms to such concentrations of microplastics alone. Our results suggest that even very high concentrations of the microplastics tested within these studies will not be of ecological importance over acute timescales, however from these data it is not possible to

predict how this relates to other types and sizes of microplastics. Where very high (unrealistic) concentrations of microplastics are used in future studies, this approach must be justified as being worthwhile and valuable for our understanding of microplastics as a pollutant. For example studies should seek to understand mechanisms of toxicity or chemical transfer processes, across a range of different polymers, particle sizes, ages and additive chemicals, where subtle effects may be difficult to interpret at lower concentrations (Huvet et al., 2016; Kuhn et al., 2018). It has been suggested that more realistic, chronic exposures could induce subtle, sub-lethal effects leading to longer-term ecosystem consequences (Au et al., 2015; Jaikumar et al., 2019; Redondo-Hasselerharm et al., 2018). While effects over chronic timescale are not always seen (Bruck and Ford, 2018; Weber et al., 2018a), such studies are likely to be more ecologically relevant than analysing acute responses to high concentrations of microplastics. Given that microplastics will not degrade quickly, environmental exposures are likely to be long-term (i.e. months, years or even decades). Therefore, experimental exposures in the order of weeks or even months would allow for the investigation of the potential sub-lethal or multigenerational effects of microplastics. It should be noted that even if the input of plastics to the environment were halted, environmental concentrations will continue to increase due to the degradation of existing plastics. Therefore it is reasonable to assume that organisms will be exposed to ever-increasing concentrations of microplastics and nanoplastics (Mattsson et al., 2015). This must be therefore taken into consideration when thinking about future worst-case scenarios and risk assessment.

If effects of microplastics and adsorbed chemicals on organisms are to be seen, it is expected that they would have been observed within a pristine, controlled setup, such as those used in the studies presented with **Chapters 5 and 6**. The lack of effects therefore imply that within the environment where geological, chemical and biological processes are more complex, the interactions between the organisms and pollutants investigated in these experiments are likely to be insignificant. In addition to the chemicals that may associate with microplastics once they enter the environment, many plasticiser chemicals are incorporated into plastics during manufacture, which are not chemically bound to the polymer structure and will leach out of the plastic over time (Geiss et al., 2009; Godwin, 2011). While hydrophobic organic chemicals (HOCs) are widespread within the environment, plasticisers would not be widely present in the absence of plastics. Given the negligible microplastic-facilitated toxicity of externally associated HOCs seen in the research presented within this thesis, it is likely that plasticisers will become significantly available to organisms as a result of microplastic presence, due to

leaching (Devriese et al., 2017; Lohmann, 2017). Plasticisers have been proven to leach out of common plastic products and cause toxic effects to aquatic organisms (Lithner et al., 2009; Lithner et al., 2012). It has been suggested that leaching of plasticisers from microplastic particles is likely to be size-dependent and as such has further toxicological implications when considering the degradation of particles within the environment (Coffin et al., 2019). However, research in this area is still limited and therefore in future studies it would be recommended to prioritise the investigation of plasticiser toxicity (including rates of leaching and effects of weathering) over the investigation of sorbed organic chemicals.

In order to effectively develop our understanding of the effects of microplastics within the environment, it is especially important that future research looks to better design experimental studies to make them relevant and relatable to predicted or actual environmental conditions, concentrations and particle transformations (Kuhn et al., 2018; Lenz et al., 2016). Due to methodological limitations, we are currently unable to understand fully the range of microplastics present within the environment, especially with respect to the lower size limit of particles present (Huvet et al., 2016). The lower particle size limit which can currently be simultaneously quantified and analysed to polymer type (using micro-FTIR imaging) is approximately 10-20 μm (Liu et al., 2019; Mintenig et al., 2019; Simon et al., 2018). It is assumed that plastic will undergo a continual degradation from macroplastic to microplastic, eventually degrading to nanoplastics (Mattsson et al., 2015). However, analytical techniques for detecting nanoplastics within environmental samples are in their infancy (Nguyen et al., 2019; Schwaferts et al., 2019). Therefore recommendations cannot easily be made for monitoring, and thus regulation of plastic particles < 10 μm. Within laboratory assays, this also limits the ability to trace particles, thus impeding understanding of toxicological mechanisms. Recent developments in producing novel, metal-doped nanoplastic particles could provide a solution to these detection limitations, *i.e.* plastic particles containing a rare metal component allowing for analysis of the metal as a proxy for the presence of the nanoplastics (Mitrano et al., 2019).

If, and when, environmental regulations for microplastics are implemented, it would be recommended to use the risk assessment approach of comparing predicted environmental concentrations to ‘ecologically acceptable concentrations’, as is often the case with chemical regulation (Crane and Giddings, 2004; Hommen et al., 2010; Rico et al., 2016). Efforts have been made to synthesise and interpret existing microplastic data on exposure and hazard by Adam et al. (2019) and Burns and Boxall (2018). These reviews highlight not only the

variability in responses between different species, but also within species, where responses (for example NOEC based on particle concentration) can be orders of magnitude different, even when exposed to the same shape and type of polymer. This may be due to different exposure conditions (for example different culture conditions or particle sizes) but can still occur even where all other exposure conditions appear to have been the same (Adam et al., 2019; Martins and Guilhermino, 2018; Rehse et al., 2016). Where species have been the subject of multiple studies, it is therefore possible to make better estimates of responses to microplastics, although it is still necessary to understand the full range of exposure conditions, in order to be able to determine the factors influencing any differences seen. Although substance regulations are usually based on the risk assessment framework, where concerns are high this type of review may be bypassed. For example, this year the European Chemicals Agency (ECHA) have proposed a restriction on all 'intentionally added microplastics' as a precautionary measure, despite little substantial evidence of harm, (ECHA, 2019). This restriction proposal aims specifically to restrict or regulate the use of microplastics added to products where their use will result in direct or indirect release to the environment. This will include products not previously regulated for microplastics including, for example, detergents, sunscreens, paints and seed coatings. Depending on the product, this may require a restriction on placing the product on the market (where microplastics will certainly enter the environment as a result of using the product), specific labelling requirements (to minimise releases to the environment where this may occur indirectly during use) or improved reporting requirements to improve the standard of information available to consumers. While this only applies to primary microplastics and does not cover the more substantial inputs from secondary sources, this could still prevent ~36,000 tonnes microplastics entering the environment annually (ECHA, 2019).

It is important to be aware that the pristine particles often used within laboratory exposures are not representative of those found within the environment. Plastics are complex heterogeneous mixtures of polymers and chemicals and as such, it is not feasible to predict microplastic effects based solely on limited studies of homogenous polymers and particle types. Laboratory studies must therefore be designed effectively to enable the determination of toxicological effects of plastics with well-defined characteristics, based on size, shape, polymer type, externally associated chemicals and weathering, to better determine the specific factors leading to any toxic effects seen (Kuhn et al., 2018; Rochman et al., 2019; Vroom et al., 2017). Given the potential for environmental transformation to significantly influence the toxicity of particles, this requires a greater understanding of these particle characteristics and transformations within

the environment. This is an area of research that has been extensively studied for engineered nanomaterials, but less is understood about how these processes will influence microplastic toxicity (Schultz et al., 2015; Syberg et al., 2015). Microplastic research is extremely multi-disciplinary, spanning ecotoxicology, microbiology, chemistry, geography, hydrology and more, and therefore it is essential that researchers collaborate to address these key questions.

The findings in presented this thesis have contributed to our understanding of microplastics as a pollutant within freshwater systems, having particular impact within the UK. This has enabled dialogue with UK regulators including the Department for Environment, Food and Rural Affairs (Defra) and the Environment Agency, regarding necessary future research and possible implications for policy and regulation around plastic items and microplastics. Microbeads within wash-off cosmetic products were banned in the UK from 2017 following a public consultation (Defra, 2016). The proposal for microplastic restriction by ECHA (ECHA, 2019) will apply to a far greater range of products and thus will be much more difficult to implement. While these restrictions are helping to prevent some microplastics entering the environment, it must be borne in mind that primary microplastics such as microbeads and glitter are only infrequently found within environmental samples. Instead the majority of particles in fact consisting of secondary microplastics formed by the degradation of larger items (Anderson et al., 2017; Boucher and Friot, 2017; Ryan, 2015). The degradation processes leading to this fragmentation are not well defined in the context of the many different polymer types present within the environment. Developing a sound understanding of these processes and their implications for conventional polymers, in addition to the relatively recent development of degradable and biodegradable polymers, will be a great challenge for scientists, regulators and industry alike (Napper and Thompson, 2019).

7. Outlook

When considering microplastics it is important to remember that microplastics form but one small part of the plastic issue. Given that microplastics usually derive from the breakdown of macroplastics, and will further degrade to form nanoplastics, it is not reasonable to consider microplastics as a separate entity (Blair et al., 2019; Lambert and Wagner, 2016). Rather, microplastics should be considered within the bigger picture of plastic manufacture, use and disposal, as a wide range of different materials from various sources (Rochman et al., 2019). By the time microplastics reach the environment, it is almost certainly too late for mitigation

or removal, due to their small size and their ability to be transported large distances (Allen et al., 2019; Horton and Dixon, 2018). Thus if any potential effects or implications of microplastics in the environment are to be avoided, they must be prevented from entering or forming within the environment in the first place.

In response to the growing global recognition of plastics as a widespread and persistent environmental pollutant, a number of small and large-scale initiatives are in place. These include, for example, individuals changing their plastic use habits, producers using natural or recycled packaging materials, microplastic removal devices such as filters or laundry bags design to capture fibres, and the development of bioplastics and degradable plastics. However, the benefits and disadvantages of novel products or plastic alternatives are not well understood by consumers due to the lack of specific definitions and regulations regarding these products. One example is that of biodegradable plastics: a common misconception is that biodegradable plastics can be thrown anywhere in the environment and will rapidly degrade to form harmless, natural materials. However, research has shown that the majority of products labelled degradable and biodegradable will not mineralise within the environment under any meaningful amount of time (Napper and Thompson, 2019). Further confusion arises from the labelling of such plastics, with terms including ‘oxodegradable’, ‘biodegradable’ and ‘compostable’, all inferring that the product will fully degrade but in reality, many such items have differing properties and will often degrade only under very specific conditions (Lambert and Wagner, 2017).

Many plastics are also theoretically recyclable, for example polyethylene terephthalate (PET), although whether they are in fact recycled depends on the waste management practices within the country or region (Eriksen et al., 2019). Across much of Europe, for example, the waste management infrastructure is unable to handle the amount of recyclable waste produced and much of this is shipped to Asian countries (Brooks et al., 2018). Here some of it will be recycled, but much of it will also end up being (often illegally) incinerated or landfilled (Ray, 2008). Where plastics are mechanically recycled, the majority of recycling practices lead to ‘downcycling’ whereby the product becomes less pure and is used to create lower-quality products with each cycle, ultimately becoming a product which requires landfill or incineration (Rahimi and García, 2017). Alternative options for recycling exist, for example pyrolysis (also known as thermal cracking) to break the polymer down to its constituent monomer components. However, further work is needed to assess the economic viability and large-scale feasibility of such processes (Brems et al., 2012). It has been suggested that ‘problem’ plastics,

which cannot be readily recycled and often end up as contaminants within the environment, such as PVC and expanded polystyrene packaging, should be phased out (WRAP, 2019). As such, in order to effectively design a product for that can be recycled, provisions must be made for the product's end of life during the design and manufacture stage. While the general public can influence the decisions and practises of businesses based on demand, ultimately it is the manufacturers and large organisations who can address the plastic pollution problem, based on the materials they use and the products they design.

Despite the recognition that plastic waste management and pollution is an increasing problem, the manufacture rate of plastics continues to increase and is projected to increase ~400% by the year 2050 (World Economic Forum, 2016). In order to prevent the continued accumulation of plastic within the environment, and any resulting harmful effects, global discussions and collaborations are needed to tackle the problem from all angles. This includes a better understanding of the sources and fate of plastics, the degradation and behaviour of traditional plastics and their alternatives, and the toxicity and long term ecological effects of the wide variety of different particle shapes, sizes and polymer types. With such knowledge we can inform future regulations, mitigations and solutions to what is indisputably one of the most prominent environmental issues today.

8. Conclusions

In recent years, efforts in microplastic research have increased significantly. This PhD research was carried out at a time when there was little knowledge of microplastic inputs, presence within the environment, availability or toxicity to freshwater organisms. The research presented within this thesis has enhanced our knowledge across these areas, and although many questions remain, these questions have become better refined and informed. In order to progress further our understanding of microplastics as an environmental pollutant, it will be essential to coordinate research efforts in understanding both the fate and effects of microplastics within the natural environment, combined with hazard studies determining toxicity and effects thresholds over realistic exposure scenarios and timescales.

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